Establishing a high throughput method for medium optimization – a case study using Streptomyces lividans as host for production of heterologous protein

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Poster 1.2.05

Use of aerobic/anaerobic system for nutrient removal (C, N, P) in dairy wastewater plant: application of RSM in batch conditions

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The batch systems appears today as a robust technology and is widely used for dairy wastewater treatment. Combined (anaerobic/aerobic) biological treatment methods for simultaneous removal of carbon, nitrogen and phosphors are discussed. The experimental design was face centered-central composite design (FC-CCD) with four operational variables (MLSS, COD:N:P ratio, aeration time and cycling time). Nine dependent parameters as the process responses were studied. The removal efficiency of COD, TKN, PO_4^{3-}, NO_3^- and amount of SVI, MLSS, MLVSS, effluent NO_3^- and effluent TN were 98.95%, 85.6%, 98.34%, 99.99%, 44.03 ml/g, 5646.97 mg/l, 4435.99 mg/l, 0.03 mg/l and 56.55 mg/l, respectively. At last, the optimum conditions for nutrients removal were found to be a level of desirability of 0.995 at MLSS 4499.84 mg/l, COD:N:P ratio 100:24.98:9, Aeration time 40 min/h and cycling time 30.11 h. At these optimized conditions, responses were shown: COD 88.44%, TKN 70%, PO_4^{3-} 87.41%, NO_3^- 82.22%, NO_3^- effluent 1.81 mg/l and TN effluent 76.81 mg/l, respectively. These results suggest significant promise of this approach for application to wastewater with high nitrogen, phosphors and carbon content in dairy wastewater plants.

Keywords: Nutrient removal percentage; Dairy wastewater treatment; Batch systems; RSM; Optimization

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Poster 1.2.06

Benzoic acid fermentation from starch and cellulose via a plant-like β-oxidation pathway in *Streptomyces maritimus*

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Benzoic acid is one of the most useful aromatic compounds. Despite its versatility and simple structure, benzoic acid production using microbes has not been reported previously. *Streptomyces* are aerobic, Gram-positive, mycelia-forming soil bacteria, and are known to produce various kinds of antibiotics composed of many aromatic residues. *S. maritimus* possess a complex amino acid modification pathway and can serve as a new platform microbe to produce aromatic building-block compounds. In this study, we carried out benzoate fermentation using *S. maritimus*. In order to enhance benzoate productivity using cellulose as the carbon source, we constructed endo-glucanase secreting *S. maritimus*. After 4 days of cultivation using glucose, cellobiose, or starch as a carbon source, the maximal level of benzoate reached 257, 337, and 460 mg/l, respectively. *S. maritimus* expressed β-glucosidase and high amylase-retaining activity compared to those of *S. lividans* and *S. coelicolor*. In addition, for effective benzoate production from cellulosic materials, we constructed endo-glucanase-secreting *S. maritimus*. This transformant efficiently degraded the phosphoric acid swollen cellulose (PASC) and then produced 125 mg/l benzoate.

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Poster 1.2.07

Establishing a high throughput method for medium optimization – a case study using *Streptomyces lividans* as host for production of heterologous protein

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Actinomycetes are widely known for production of antibiotics, though as hosts for heterologous protein expression they show great potential which should be further developed. *Streptomyces lividans* is especially interesting due to very low endogenous protease activity and the capability to secrete proteins to the medium. As saprophyte it also has the ability to use a very diverse range of substrates including cellulose. Furthermore, a growing array of genetic tools has been developed, while sequencing and annotation is still to follow in the near future as various initiatives are in progress.

Medium composition can have great effect on the cellular performance, in particular on heterologous protein production. It is a parameter that can be adjusted regardless of GMO concerns or knowledge of genomic sequence. Optimizing medium composition can be labor intensive opening up for introducing automation.

In this study a potential high throughput method was tested for optimizing medium composition, with respect to nitrogen, to improve heterologous protein production in *S. lividans*, using mRFP as a model protein. A large number of nitrogen sources were tested in an initial, highly automated, screen. Subsequently the most promising candidates were tested in milliliter scale, followed by final verification in lab-scale fermentation. The method has the great advantage that the initial steps have a high degree of automation, which allows to retain a relatively high number of candidates. A further benefit of this approach is that substantial physiological knowledge is gained from the unsequenced model producer.

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